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Anti-malignin antibody in serum and other tumor marker determinations in breast cancer

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Abstract

In this study, 154 healthy volunteers and 76 patients were tested using the anti-malignin antibody in serum (AMAS) test. Of the 154 volunteers, three were AMAS positive. After further examination, two were positive for cancer and one had a history of ulcerative colitis. Tumor biopsies of 43 suspicious mammography patients revealed that 32 were cancerous and 11 were benign by pathology. For the cancer patients, 31/32 were positive for the AMAS test, while 4/11 of the pathological benign cases were AMAS positive. In comparison to cancer antigen tests, AMAS was more sensitive (97%) in detecting breast cancer than CEA (0%), CA 15-3 (10%), CA 19-5 (5%) or CA 125 (16%) in the same patients.

Keywords: Anti-malignin antibody in serum test (AMAS); Mammogram; Screening; Monitoring; Tumor markers; CA 125; CA 19-9; CA 15-3; Carcinoembryonic antigen

1. Introduction

According to the American Cancer Society, almost 563 000 people in the USA will die of malignancy, of whom almost half could have been saved by early diagnosis and prompt treatment [1]. The results from a national survey indicate that Americans are not making optimal use of tests for the early detection of cancer [2].

While the mammogram is now recommended for routine screening of breast cancer, it is an insufficient screening procedure. There is a high percentage of false positives, and the false negative rate is difficult to determine. Additionally, women with silicon implants in their breasts may not be candidates, the reading of a mammogram is subjective, and only a small percentage of women are screened by mammography [2]. Less than 40% of women older than 40 years of age have ever had a mammogram [2]. This is due to a concern for cumulative effects of radiation, pain in obtaining representative images, the inconvenience of having to make a separate appointment outside the physician's office, and the expense.

New diagnostic tests are needed to complement mammograms in order to help differentiate early stage cancer from benign disease [3]. Current procedures involve biopsying a suspicious lump or calcified region. A less invasive procedure, such as a serum assay, would be of value in assessing whether a suspicious mammogram represents malignancy. In this report, tumor marker assays were performed along

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with biopsies following a suspicious mammogram in order to determine if tumor markers aided in diagnosis and could be used to monitor residual disease. The serum marker tests included anti-malignin antibody in serum (AMAS), Carcinoembryonic antigen (CEA), CA 15-3, CA 19-9, and CA 125.

2. Materials and methods

2.1. Laboratory diagnosis

Quality control for AMAS involved double-blind testing. Pathologists without any knowledge of tumor marker results made the histological diagnoses. Concurrently, laboratory personnel without knowledge of the clinical findings performed the laboratory assays.

2.2. Healthy volunteers

Healthy volunteers ($n = 154$) from Baptist Hospital were used, each of whom was informed of the scientific nature of this study and consented to being controls. The volunteers ranged in ages from 25–70 years, with the majority in the 33–53 years age range (43 ± 10 years, SD) and with 52% being female. Some of the controls were monitored for over a year, and a total of 206 tests were run on the group.

2.3. Control patients with no tumor mass

Control patients included no evidence of disease ($n = 10$), microcalcifications ($n = 18$) and fibrocystic disease ($n = 5$). All control and positive patients were sequentially used in this study from Dr Derhagopian's practice.

2.4. Control patients with measurable tumor mass and negative pathology

Serum samples were obtained within 2 weeks before or after surgery on all patients with suspicious mammograms and a measurable biopsy sample of 1–50 mm ($n = 11$).

2.5. Patients with measurable tumor mass and positive for pathology

Serum samples were obtained within 2 weeks before or after the surgery ($n = 32$).

2.6. Clinical criteria

The clinical information included radiology, type of operation, primary or metastatic tumor diameter, number of positive lymph nodes out of the total analyzed, histopathology, days post surgery, and tumor marker determinations. The AMAS measurements were performed under an IRB approved protocol at Baptist Hospital.

2.7. AMAS assay

The laboratory employed the same quality-controlled procedure [4] used for all previous 3315 double-blind AMAS tests [5–7]. Blood was collected in Becton-Dickinson #6440 pink top vacutainer tubes, being the first drawn tube if there were a series of tubes. Serum separators were not used. Blood was centrifuged at 2–4°C and the serum transferred by glass Pasteur pipettes to a NUNC tissue culture tube (Thomas Scientific, Swedsboro, NJ). Serum was kept on dry ice and was assayed within 24 h of the blood being drawn. The test itself is characterized by specific immunoadsorption of the antibody from serum to TARGET™ reagent (Oncolab, Boston, MA). TARGET™ reagent is composed of malignin, a 10-kDa peptide isolated from glioblastoma cells grown in tissue culture medium. The purified malignin consists of stable composition of 89 amino acid residues (which are characterized by high glutamic acid, aspartic acid, and low histidine composition) and is covalently bound to bromoacetylcellulose beads [8,9]. An aliquot of 0.2 ml of TARGET™ reagent containing the malignin-bound bead is shaken vigorously with 0.2 ml of serum at 0–5°C, washed with cold normal saline, then shaken vigorously with 0.25 M acetic acid at 37°C to elute the bound antibody. The antibody is quantitated as protein by absorption at 280 nm and expressed in μg/ml serum [4]. Two serum samples each are incubated with the TARGET™ reagent for a fast binding (15 min, 4°C) and a slow binding (2 h, 37°C) assay. The amount bound to the slow binding minus the fast binding (non-specific binding) equals

Table 1
Summary of AMAS values for healthy volunteers, patient controls and cancer patients

Patient population	Mean	± SD	n
Healthy volunteers	76	23	154
Control patients (no tumor mass)[a]	108	21	33
Control patients (tumor mass/ negative path)[a]	137	60	11
Patients (positive by pathology)[b]	220	64	32

[a] Control patients with suspicious mammograms with benign conditions, microcalcification, or negative mammograms but history of breast cancer.

[b] Patients with suspicious mammograms were found to have cancer by pathology, tumor size ranged from 1–60 mm in diameter, except for four patients in which two had more than two nodes positive, all were stage 1.

the net-AMAS (specific binding). The terms net-AMAS and AMAS are interchangeable.

All specimens were determined in duplicate. Known amounts of IgM monoclonal anti-malignin antibody [10,11] were determined to be used as controls for each serum. Normal and elevated AMAS values are within the range of earlier studies [5–7], i.e. negative values ranging from 0–134 μg/ml, while positive determinations were equal or greater than 135 μg/ml. The range of AMAS from 100–134 μg/ml is designated as borderline but designated as normal if confirmed on a second test to be 134 μg/ml or below.

2.8. CEA, CA 15-3, CA 19-9 and CA 125 assays

In contrast to the antibody detection AMAS test, these tumor markers are cancer antigen (CA) tests. The CEA test was performed using the radioimmunoassay (RIA) kit from Hybritech (San Diego, CA). Other assays were determined by the Centocor solid phase RIA (Amersham). Normal ranges for the tumor antigen shedding marker assays were as follows: CEA, 0–3 units/ml for non-smokers, 0–10 units/ml for smokers; CA 15-3 0–35 units/ml; CA 19-9, 0–70 units/ml; and CA 125, 0–30 units/ml.

2.9. Statistical data

The means and standard deviations were determined for the control, negative AMAS, and positive AMAS groups. All patients were analyzed in a double blind data acquisition and analysis. The decay curves showing the disappearance of the anti-malignin after primary breast tumor removal were used to calculate the mean half-life of the anti-malignin antibody.

3. Results

3.1. Healthy volunteers

Table 1 shows the mean AMAS value of 76 ± 23 μg/ml for 154 healthy volunteers. Three of these controls repeatedly tested positive (>134 μg/ml). Cervical carcinoma in situ was found in one of the controls (DP), in whom a hysterectomy was performed 3 weeks (330 μg/ml) after the first AMAS reading (132 μg/ml). Six weeks after surgery, her AMAS value returned to normal levels and was monitored for recurrence (Fig. 1, DP). Another positive control (ML) was examined and found to have a deeply embedded basal cell carcinoma. The third had a 10 year history of ulcerative colitis and was monitored over 4 months during the study with borderline positive values (ML in Fig. 1). Counting this control

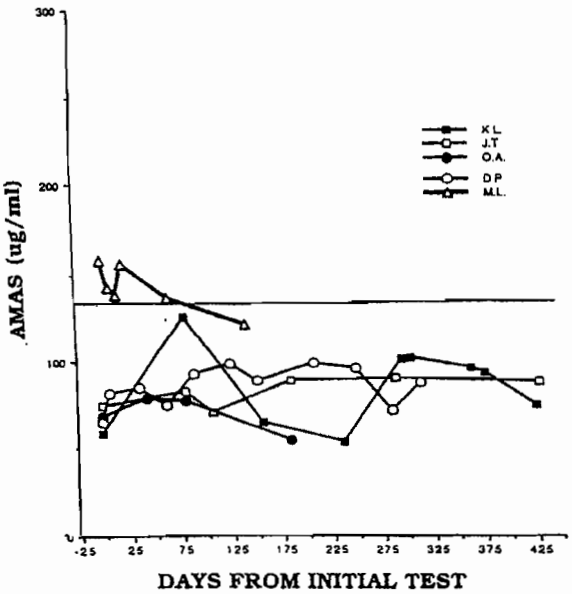

Fig. 1. Monitoring of AMAS controls.

Table 2
Analyses of control patients with tumor mass and negative for cancer by pathology

Patient code	Patient age[a]	Biopsy size (mm)	Pathology	Days post[b]	AMAS (<135)[c]	CEA (<3)[d]	CA 15-3 (<35)[d]	CA 19-9 (<70)[d] (<200)	CA 125 (<30)[d] (<40)
BC	61	8	Fibrocystic	−9S	80−	1.3−	ND[e]	ND	ND
BP	45	50	Fibrocystic	−32B	132−	ND	ND	ND	ND
EF	46	25	Benign	+90S	133−	ND	ND	ND	ND
BG2	38	15	Benign lobular hyperplasia	−13S	100−	1.4−	25−	37−	24−
JB	53	5	Fibroadenoma	+757S	94−	ND	ND	ND	ND
JS	47	13	Fibroadenoma	+18S	105−	ND	ND	ND	ND
ML	50	2	Fibroadenoma	+34B, +44B	100−	ND	ND	ND	ND
CC	44	7	Fibroadenoma	−12B, +39B	147+	ND	ND	ND	ND
NL	52	15/8	Bilateral fibroadenoma	+1S	153+	0.1−	39−	5−	12−
LK	ND	30	Fibroadenoma	−21B	162+	ND	ND	ND	ND
CC2	43	30	Proliferative fibrocystic disease	−21B	298+	ND	ND	ND	ND

[a] At the time of mammography.
[b] Breast cancer surgery (S) or biopsy (B).
[c] (μg/ml).
[d] (units/ml).
[e] ND, no data.

as a false positive, the false positive rate thus far is 0.7% (one out of 154 volunteers).

Fig. 1 illustrates the precision of the AMAS test in control populations over a long period. Four control subjects, JT, KL, OA and DP, showed consistent AMAS values up to 425 days. None of the positive controls with the AMAS test were positive for the other tumor markers reported in this study.

3.2. Control patients with no tumor mass

Table 1 shows data from 33 control patients who had no tumor mass, consisting of those with no evidence of disease (ten), with microcalcifications (18), and fibrocystic disease (five). The mean AMAS value was 108 ± 21 μg/ml and is significantly below the positive value of 135 μg/ml [4–11].

3.3. Control patients with tumor mass and negative by pathology

Tables 1 and 2 show the analyses of 11 patients with positive mammograms but negative for cancer by pathology with a mean AMAS value of 137 ± 60 μg/ml. As shown throughout this paper, the pathology examination consisted of representative sections processed. There were four patients, three with fibroadenomas and one with proliferative fibrocystic disease, who had elevated AMAS values. One of these patients had a history of multisite malignant tumors. The overall false positive rate for the control patient groups is 9% (4/44) on first determination only. Failure to process the entire tissue may have prevented the revealing of a small in situ lesion. Repeat determinations, previously reported to decrease the false positive rate to less than 1% [4–6], were not performed in this study.

3.4. Patients positive for cancer by pathology

The results from 32 suspicious mammograms and positive diagnoses, are shown in Tables 1,3, and 4. In Table 3, there was no lymph node involvement in 85% (24/28 excluding four with no data) of the patients. The mean AMAS value of 220 ± 64 μg/ml (Table 1) was well above the published minimum positive value of 135 μg/ml [4–11].

3.5. Comparisons between the tumor markers

Table 4 shows a comparison among the various tumor markers. The breast cancer patients were 97% positive by the AMAS test (Table 3). In contrast, the other tumor markers performed poorly, only being 0–16% positive for the cancer antigen tests. The one patient (NB) who was negative by AMAS but positive by pathology (which represents a false negative rate of 3.1% (1/32)), had a 15 mm infiltrating ductal carcinoma with a positive CA 125 level. There was no other evidence of residual cancer in this patient. The repeat AMAS was still negative (107 μg/ml) 123 days after surgery, while the CA 125 was still positive (data not shown).

In Table 3, the AMAS test is shown to be sensitive to small primary tumors and carcinoma in situ. All 11 patients with 10 mm or less diameter tumors were positive for the AMAS test.

3.6. Monitoring for the presence of residual disease with the AMAS test

Figs. 2 and 3 show curves of the changes in the AMAS value after primary tumor removal. Based on the data in Fig. 2, the half-life of the anti-malignin was determined to be approximately 6 weeks. Interestingly, higher initial AMAS values near surgery, resulted in a greater decay slope in general. A significant decrease in AMAS can occur within 1 week after primary tumor removal. A drop in the AMAS value after primary tumor removal is believed to be due to the absence of residual tumor with subsequent decrease in the IgM malignin antibody level in the serum.

Conversely, elevated anti-malignin values after surgery may indicate the presence of residual tumor [29,30]. In Fig. 3, the post-surgery AMAS values are shown for the four patients who continued to have positive AMAS after surgery. Patient SB had a dramatic rise in her AMAS. Three months after the last reading, she was diagnosed as having malignant lymphoma, large cell type. Patient CM still had an elevated AMAS 36 days after surgery. Her AMAS value decreased and has remained negative. Patient PD, after removal of a 60 mm intraductal carcinoma with 17 lymph nodes negative, showed a decrease initially then significant increase from day 40 to day

Table 3
Analyses of patients with measurable tumor mass and positive by pathology

Patient code	Patient age[a]	Operation type	Size (mm)	Nodes (+/total)	Pathology/surgery[b]	Time between mammogram and surgery (days)	Days AMAS to surgery	AMAS (<135)[c]	CEA (<3)[d,e] (<200)	CA 15-3 (<35)[d] (<40)	CA 19-9 (<70)[d]	CA 125 (<30)[d] (<200)[d]
AAf	75	Mastectomy	8	0/30	Intraductal	18	+1	302+	0.2−	12−	5−	12−
AW[i]	75	Modified radical mastectomy	15	0/22	Infiltrating poorly differentiated ductal	227	+2	233+	2.7−	44+	71+	12−
AW2	56	Left mastectomy	16	0/26	Poorly differentiated ductal	64	−49	175+	ND[g]	ND	ND	ND
BB2	40	Right breast	14	0/31	Infiltrating ductal poorly differentiated	66	+3	310+	ND	ND	ND	ND
BG	63	Mastectomy	12	None[h]	Infiltrating ductal	13	+2	172+	ND	ND	ND	ND
CC3	ND	Left mastectomy	12	1/26	Infiltrating ductal	ND	−11	156+	ND	ND	ND	ND
CJ	75	Left mastectomy	40	2/13	Infiltrating ductal	50	+7	195+	ND	ND	ND	ND
CK	ND	Left mastectomy	15	1/26	Infiltrating ductal	~ 60	−11	156+	ND	ND	ND	ND
CM[i]	47	Modified radical mastectomy	10	0/36	Infiltrating poorly differentiated ductal	54	+7	246+	0.2	16−	8−	18−
EG[i]	47	Right biopsy	23	0/22	Infiltrating poorly differentiated ductal	16	+1	238+	ND	11−	5−	7−
EG2	77	Right breast mastectomy	23	0/22	Infiltrating poorly differentiated ductal	16	+8	238+	ND	ND	ND	ND
EB4	42	Left breast biopsy	9	0/22	Infiltrating poorly differentiated ductal	46	+19	252+	ND	ND	ND	ND
EP[i]	69	Nodule	5	ND	Infiltrating ductal	39	+7	175+	4.1−	15−	21−	0.7−
FB	ND	Left breast	9	ND	Infiltrating ductal poorly differentiated	ND	+19	252+	ND	ND	ND	ND
EH	70	Left breast biopsy	45	None[h]	Intraductal modified differentiated	58	+62	157+	ND	ND	ND	ND
HH[i]	ND	Node	13	7/29	Metastatic adenocarcinoma	18	+1	345+	0.9−	19−	5−	9−
JF[i]	39	Left mastectomy	13	0/15	Intraductal	16	−10	203+	0.7−	16−	7−	3−
LM[i]	40	Mastectomy	50	0/19	Lobular infiltrating	241	+2	274+	1.0−	16−	16−	7−

Table 3 (*continued*)
Analyses of patients with measurable tumor mass and positive by pathology

Patient code	Patient age[a]	Operation type	Size (mm)	Nodes (+/total)	Pathology/surgery[b]	Time between mammogram and surgery (days)	Days AMAS to surgery	AMAS (<135)[c]	CEA (<3)[d,e] (<200)	CA 15-3 (<35)[d] (<40)	CA 19-9 (<70)[d]	CA 125 (<30)[d] (<200)[d]
MB2[f]	83	Right mastectomy	6	0/12	Infiltrating with differentiated tubular	7	+1	283+	2.7−	21−	33−	46+
MC[f]	ND	Breast	50	0/18	Infiltrating ductal	8	−5	252+	1.4−	31−	5−	10−
MC3	61	Left mastectomy	5	0/18	Multiple sites, no pathology	66	−4	240+	ND	ND	ND	ND
NB[f]	50	Excisional biopsy	15	ND	Infiltrating ductal	21	+18	119−	0.3−	35−	11−	59+
ND	48	Left breast	35	0/25	Intraductal	129	+2	156+	ND	ND	ND	ND
NR	53	Right breast	2	0/23	Intraductal comedo	96	−4	247+	ND	ND	ND	ND
NW[f]	36	Simple mastectomy	6	0/18	Infiltrating ductal poorly differentiated	8	+5	393+	0.2−	36+	39	14−
PD[f]	47	Simple mastectomy	60	0/17	Intraductal	15	−7	206+	2.1−	19	66−	16−
PS[f]	50	Left mastectomy	3	0/04	Intraductal in situ	ND	+1	194+	ND	17−	18−	66+
RJ[f]	59	Mastectomy	31	0/28	Infiltrating moderately well differentiated	7	+4	217+	ND	26−	1−	11−
RL[f]	47	Lump/ modified radical mastectomy	17	0/22	Infiltrating moderately differentiated	18	−16	158+	3.1−	24−	39−	20−
RO[f]	43	Left breast	10	0/26	Infiltrating lobular carcinoma	15	−12	170+	1.6−	29−	5−	13−
SB2[f]	33	Modified radical mastectomy	12	0/37	Infiltrating ductal	ND	+1	183+	1.7−	17−	22−	20−
VM[f]	45	Left biopsy	1	ND	Intraductal	57	+13	146+	1.0−	10−	4−	11−

[a] Age at time of surgery.
[b] Most patients measured within 15 days of surgery.
[c] Normal value in μg/ml.
[d] Normal values in units/ml.
[e] Smokers (< 10 units/ml).
[f] Data of other tumor markers included.
[g] ND, no data - or not determined.
[h] None, path report stated 'none' of the lymph node were positive, no numbers given.

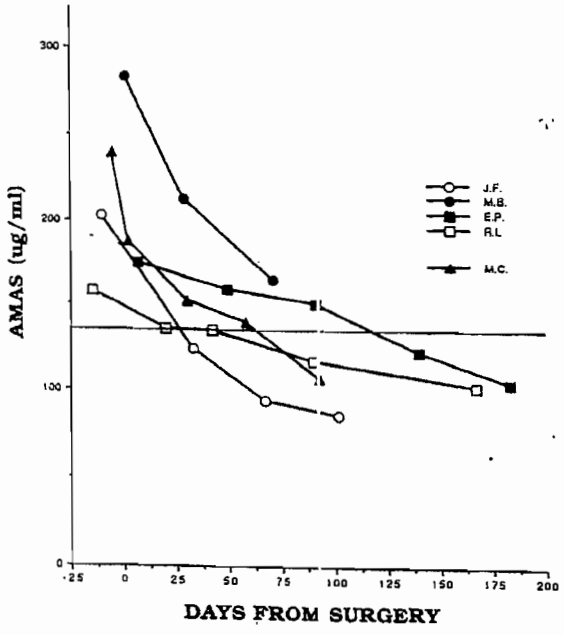

Fig. 2. Monitoring of primary breast cancer patients post surgery using the AMAS test.

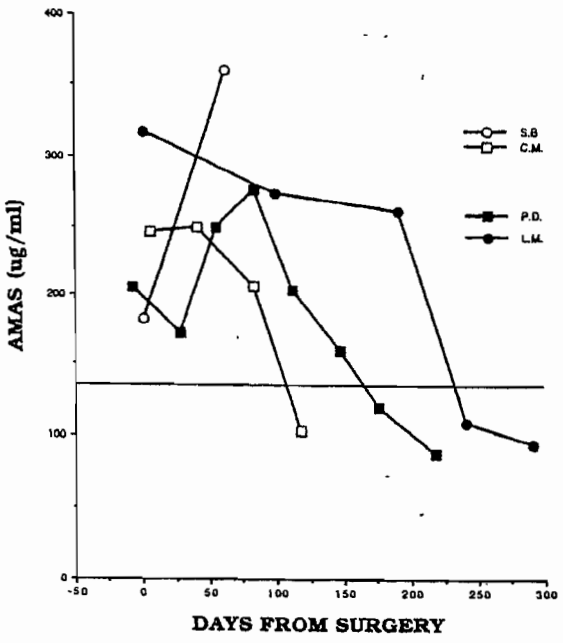

Fig. 3. Patients with elevated AMAS values after surgery

80 after surgery. At day 80, breast and facial cosmetic surgery was performed. Subsequently, the AMAS decreased significantly over the next 60 days to a control level. This rise and fall of AMAS for this patient is unclear. Patient LM had an elevated AMAS level which finally decreased to the control levels during the study.

4. Discussion

The AMAS test measures serum levels of Anti-Malignin, an antibody found to be elevated in most patients with a wide range of malignancies. The data from Bogoch et al. [4,5,7–11,30], independent studies performed by SmithKline Laboratories [6], by Abrams et. al. [12], Thornthwaite [12,29], and this study collectively demonstrate that anti-malignin is elevated almost regardless of site or cell type of the malignancy. For sera determined within 24 h of being drawn, the false positive and false negative rates are less than 1% (specificity and sensitivity greater than 99%) in 3315 double-blind tests of patients and controls [4–6].

This test measures levels of an antibody instead of measuring antigen. Anti-malignin is the antibody to malignin, a 10-kDa polypeptide which has been found to be present in the cell membrane of the most malignant cell types [4–11]. The AMAS test measures a well-defined antibody whose serum levels rise early in the course of the disease. In some cases, the AMAS test has been positive (elevated) early, i.e. up to 19 months before clinical detection [4–12].

The CEA and CA tumor marker assays performed poorly, possibly because the stage 1 cases were too early to permit detection of sufficient antigen shedding into serum. Tests, such as CEA [13], measure

Table 4
Comparisons between the tumor markers for the initial samplings of breast cancer patients[a]

Tumor marker	MEAN ± SD	% Positive	n
AMAS	220 ± 64 μg/m	97	32
CEA	1.4 ± 1.1 units/ml	0	16
CA 15-3	22 ± 9 units/ml	10	19
CA 19-9	20 ± 20 units/ml	5	19
CA 125	20 ± 20 units/ml	16	19

[a] Normal ranges: AMAS <135 μg/ml; CEA <3 units/ml; CA 19-9 <70 units/ml; CA 125 <30 units/ml; CA 15-3 <35 units/ml.

less well-defined antigens in which serum levels tend to be inconsistent and elevated only late in the disease. None of the tested patients ($n = 16$) were positive for CEA. For CA 15-3, only 65% of metastatic breast cancer patients and only 10% of patients with primary breast cancer were positive by this test [14–19]. The CA 19-9 antigen is identified by a monoclonal antibody developed by Koprowski et al. [20]. While the highest average CA 19-9 levels have been reported in pancreatic and hepatobiliary cancers, there was only a positive predictive value in pancreatic cancer of 54 [21] and 67% in hepatobiliary cancer [22,23]. The test may show elevations in gastric and colorectal cancers [23]. Our study showed only 10 and 5% of the breast cancers were positive for CA 15-3 and CA 19-9, respectively. The CA 125 assay measures the serum level of a 200 000 Da glycoprotein (OC 125) expressed by derivatives of coelomic epithelium [24–28]. This study showed only 16% of the breast cancer patients were positive for CA 125.

Except for the healthy volunteer population, this paper does not address the utility of the AMAS test for screening. For effective screening, several criteria should be satisfied. The test must be common, serious, economical, acceptable, valid, reliable, repeatable, specific, sensitive and risk free. From data on the healthy volunteers and patients, the AMAS test has been shown to be a very reliable test with a high degree of precision for the controls and breast cancer patients we have studied to date [29,30].

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